Selection for verticillium wilt resistance in potato breeding populations derived from four cross types

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Abstract

Verticillium wilt (VW) is one of the important yield-limiting diseases for potato production. To develop resistant clones, the potential for early generation selection was studied using three basic selection methods, individual, family, and within family selection, for two clonal generations. A total of 152 clones were derived from four cross types (2x × 2x, 2x × 4x, 4x × 2x and 4x × 4x). Clones were evaluated for maturity, symptom expression, yield and stem colonization in replicated trials. Heritability and selection response for the traits were estimated for each selection method. Direct selection in the second clonal generation and individual selection showed more gain than that from other methods. Both 2x × 2x and 4x × 2x families were higher yielding and had lower stem colonization scores than 2x × 4x and 4x × 4x crosses. Therefore, 2x × 2x or 4x × 2x crosses between carefully chosen parents with high yield and VW resistance may produce offspring with superior performance.

Key words: potato (*Solanum tuberosum*) — early generation selection — verticillium wilt — family selection — 4x × 2x

The soil-borne fungus *Verticillium dahliae* Kleb is the main causal agent of verticillium wilt (VW) in temperate potato-growing regions. It is a widespread disease, causing yield losses of 10–50% (Rowe and Powelson 2002). Soil fumigation has been effectively used to control VW but it is expensive and results in negative environmental effects. Host plant resistance offers the most cost-effective and long-term control. At present, six of the seven major cultivars in primary production regions in the United States are susceptible; only 'Ranger Russet' is moderately resistant to VW. Breeding for VW resistance is especially challenging due to ambiguous symptom expression, a lack of high-throughput assessment tools, a strong environmental component to disease expression and high stem-to-stem variation for pathogen populations in infected plants (Davis et al. 1983, Fradin and Thomma 2006, Frost et al. 2007). Therefore, more effective and efficient breeding methods must be explored.

Early generation selection has been proven to improve breeding efficiency for traits such as chipping quality and disease resistance in potato (Jellis et al. 1986, Thill and Peloquin 1995, Bisognin and Douches 2002, Hayes and Thill 2002, 2003). A major advantage of this technique is that it allows for selection while population sizes are large, with more genetic variability than in later generations. In order to effectively carry out early generation selection, it is important to focus on traits with high heritability estimates (Maris 1988, Gopal et al. 1992, Love et al. 1997). Resistance to VW in potato has been reported to be heritable and stable from generation to generation (Hunter et al. 1968, Corsini et al. 1985, Hoyos et al. 1993, Concibido et al. 1994, Jansky et al. 2004).

While conventional potato breeding focuses on 4x × 4x crosses, unilateral sexual polyploidization (USP) using 2n gametes is advantageous. Due to a triploid block in potato, crosses between tetraploids and diploids will yield tetraploid offspring if the diploid parents produce 2n gametes. Numerically unreduced (2n) gametes result from meiotic mutations that produce the genetic equivalent of either first-division restitution (FDR) or second-division restitution (SDR) (Carputo et al. 2000). 2n eggs in potato are produced by an SDR mechanism (Werner and Peloquin 1987) and most 2n pollen is produced by FDR, so 4x × FDR 2x crosses are expected to differ from SDR 2x × 4x crosses. Buso et al. (1999) demonstrated that USP with FDR 2n pollen is superior to 4x × 4x crosses for generating a high percentage of high-yielding clones with desirable traits from small families. In addition, high-yielding clones from 4x × 2x crosses often exhibit environmental stability (Darmo and Peloquin 1990). Valuable genetic diversity from wild diploid relatives is effectively transferred into the cultivated potato via USP (Darmo and Peloquin 1990, Jansky and Peloquin 2006). The USP scheme appears to be especially valuable for the incorporation of VW resistance, as diploid wild relatives offer stronger sources of resistance than tetraploid potato cultivars. Frost et al. (2006) reported that VW resistance from diploid hybrids is effectively transferred to the 4x level via 4x × 2x crosses. Therefore, early generation selection for VW resistance may be more effective in progeny from 4x × 2x crosses than in those from 4x × 4x crosses.

The objective of this study was to compare the efficiency of early generation (single-hill) selection for VW in populations derived from 4x × 4x, 4x × 2x, 2x × 4x and 2x × 2x crosses. These four different cross types were treated as families and then three basic selection methods (individual, among family and within family) for the first and second clonal generations of a breeding programme were compared based on heritability and selection response using the analytic procedure of Tai (1974).

Materials and Methods

Plant material: Two single-hill replications of each of 152 clones were planted at the Hancock, Wisconsin, Agricultural Experiment Station on two *V. dahliae*-infested fields on 12 May 2004 for the first clonal generation. On 9 May 2005, for the second clonal generation, three four-hill replications of each of the 152 clones were planted on one of the *V. dahliae*-infested fields used in the single-hill experiment. Clones were planted in a randomized complete block design with 60 cm within-row spacing for the single-hill trial and 30 cm within-row spacing for the four-hill trial. Plants were grown using best management practices. Families were chosen to represent an array of ploidy combinations (2x × 2x, 2x × 4x, 4x × 2x and 4x × 4x) and were provided by Christian Thill, University of Minnesota. The number of clones for 2x × 2x, 2x × 4x, 4x × 2x and 4x × 4x cross types was 38, 20, 40 and 54, respectively.

Evaluation: Each plant was scored for vine maturity and VW symptom expression two times during the growing season (3 August and 23 August in the single-hill trial; 24 July and 17 August in the four-hill trial). Maturity was scored on a 1 (senescent) to 5 (pre-flowering) scale. Symptoms were assessed by estimating the per cent of a plant's foliage exhibiting necrosis, chlorosis and/or wilting. Just prior to harvest, basal stem segments of primary stems were collected from each plant. They were dried at room temperature for 1 month and ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) with a 40-mesh screen. After each sample was ground, the mill was vacuumed to remove debris. A 50-mg sample from each stem was plated on a 10-cm Petri dish containing modified nutrient pectate agar (NPX) as a selective medium (Butterfield and DeVay 1977). Following a 2-week incubation period at room temperature in the dark, the number of *V. dahliae* colonies in each Petri dish was counted as a measure of stem colonization. For yield evaluation, tubers were harvested from each infested plot and total tuber weight was measured in the field.

Artificial field infection: Each plot was inoculated by spreading rye inoculum over tubers in an open furrow, and then the furrow was closed. To create the rye inoculum, rye seeds were soaked in distilled water overnight, drained, placed into bags and autoclaved twice at 121°C for 70 min each time. The V18 isolate of *V. dahliae* race 4A from severely infested potato fields was cultured in Czapek-Dox broth medium for 1 week. A 10-ml aliquot of the Vd18 conidial suspension (6×10^6 cfu/ml) was injected into each bag containing 1 kg of rye seeds. Inoculated rye seeds were incubated for 2 months at room temperature. The inoculum was then air-dried and ground in a Wiley mill with a 60-mesh screen. A 2-g aliquot of ground rye inoculum containing 10^4 cfu/g was spread over each potato seed piece at planting.

Analysis of data: Symptom expression was determined by calculating the relative area under the disease progress curve (RAUDPC). An analysis of variance was performed on each data set using the General Linear Model in SAS v.9.0 (SAS Institute, Raleigh, NC, USA). Means were separated using the least significant difference test at P = 0.05. Pearson's correlation coefficients were calculated using SAS. For the analysis of heritability and selection response, stem colony counts were transformed using $\log(n + 1)$ and then averaged over clone, and denoted as LM. Heritability was estimated in two ways as suggested by Tai (1974): standard unit and variance component. Standard unit heritability was estimated based on the analysis of variation between the first and second clonal generations. Variance component heritability was estimated based on the analysis of variance in the second clonal generation. The average number/cross type was obtained by calculating the geometric mean, which was roughly n = 36. The expected selection response (R) was calculated as $R = ih^2\sigma p$ where i is the selection intensity, h^2 is the heritability, and σp is the square root of the phenotypic variance.

Results

In both the single-hill and four-hill trials, maturity correlated with symptom expression (Table 1). In the four-hill trial, maturity also correlated with yield and stem colonization. Of interest is that stem colonization correlated with symptom expression in the four-hill, but not the single-hill, trial.

Significant correlations were detected between the single-hill and four-hill trials for each of the four cross types for all traits except stem colonization (Table 2). Only the 4x × 2x cross had a significant correlation for stem colonization between the two trials. In general, correlation coefficients between single- and four-hill generations were small for all traits. The highest correlation coefficient value (0.62) in this experiment was observed in the 4x x 2x cross for yield.

In the single-hill trial, the 4x × 4x cross had the highest stem colonization (Table 3). The 4x × 2x cross had the most resistance to VW (lowest stem colonization) and the 2x × 4x cross had the lowest yield. In the four-hill trial, the 4x × 4x cross had the highest yield and stem colonization and again the 2x × 4x cross had the lowest yield. Overall, yield and stem colonization were higher in the single-hill than in the four-hill trial.

Table 1: Pearson correlation coefficients for three traits scored in the single-hill and four-hill trials, across all family types

	Vine maturity	RAUDPC	Yield
Single-hill trial			
RAUDPC	−0.6101***		
Yield	−0.0854	−0.0171	
Stem Colonization	−0.0589	0.1642	0.1338
Four-hill trial			
RAUDPC	−0.5946***		
Yield	−0.3589***	0.0241	
Stem colonization	0.2426***	−0.3995***	0.1780*

RAUDPC, relative area under the disease progress curve.
*p = 0.05, ***p = 0.001.

Table 2: Pearson correlation coefficients between the single-hill and the four-hill trial for each cross type

	Four hill			
Single hill	Maturity	RAUDPC	Yield	SC[1]
2x × 2x				
Maturity	0.289**			
RAUDPC		0.461***		
Yield			0.283**	
SC				0.085
2x × 4x				
Maturity	0.307*			
RAUDPC		0.491***		
Yield			0.334**	
SC				−0.072
4x × 2x				
Maturity	0.194*			
RAUDPC		0.435***		
Yield			0.624***	
SC				0.215*
4x × 4x				
Maturity	0.414***			
RAUDPC		0.473***		
Yield			0.442***	
SC				0.145

RAUDPC, relative area under the disease progress curve.
*p = 0.05, **p = 0.01, ***p = 0.001.
[1]Stem colonization: cfu *Verticillium dahliae*/g dried stem tissue.

Table 3: Progeny performance of four cross types for yield and *Verticillium dahliae* stem colonization in the single-hill and four-hill trials. Standard deviations are indicated in parentheses

Cross	Single-hill		Four-hill	
	Yield/hill (kg)	SC (cfu/g)	Yield/hill (kg)	SC (cfu/g)
2x × 2x	1.91 (1.24) a	1215 (1386) b	0.85 (0.31) b	450 (670) b
2x × 4x	1.49 (0.91) b	1210 (1385) b	0.74 (0.29) c	461 (555) b
4x × 2x	1.90 (1.19) a	766 (818) c	0.87 (0.35) b	688 (1001) b
4x × 4x	2.15 (1.28) a	1701 (1936) a	0.99 (0.40) a	1037 (1223) a

SC, stem colonization.
Means in columns followed by the same letter are not significantly different using Fisher's least significant difference at α = 0.05.

Table 4: Standard unit and variance components heritability estimates for individual (i), family (f) and within-family (w) selection

Trait	Standard unit			Variance components		
	h_i^2	h_f^2	h_w^2	h_i^2	h_f^2	h_w^2
RAUDPC	0.53	0.15	0.57	0.79	0.92	0.78
Maturity	0.31	0.18	0.33	0.84	0.93	0.83
Yield	0.66	0.99	0.63	0.73	0.89	0.72
LM[1]	0.24	0.61	0.16	0.54	0.89	0.49

RAUDPC, relative area under the disease progress curve.
[1]LM: stem colonization mean, log(cfu + 1).

Heritability values from variance component estimates were larger than the corresponding standard unit estimates except for yield using family selection (Table 4). For all traits, heritabilities from the variance component estimates were highest using family selection, followed by individual and within-family selection. However, rankings of the three selection methods were different when they were based on heritability values calculated using the standard unit method. For symptom expression (RAUDPC) and maturity, the largest heritability values were obtained from within-family selection but for yield and stem colonization (LM), family selection produced the highest values. In general, standard unit estimates of heritability between the two clonal generations tested separately over 2 years were lower than those based on estimates of variance components from the second clonal generation trial (Table 4).

Table 5 provides estimates of the expected response to selection for the single-hill trial (left columns) and the four-hill trial (right columns). A selection intensity of 1.27 was used to select the 25% of clones or families. Most of the estimated gains from selection in the first clonal generation were less than those attributed to direct selection in the second clonal generation. The only exception was family selection for yield in the first clonal generation (single-hill trial) which gave a slightly better result than the corresponding direct selection in

the second clonal generation (four-hill trial). Regarding the three selection schemes, individual selection was better than others in the second clonal generation. However, for selection in the first clonal generation, a better method would be determined based on the trait. For example, in the first clonal generation, individual selection based on yield showed more gain than other selection schemes but for stem colonization, family selection was slightly better than individual selection.

Discussion

This study was carried out to compare the efficiency of early generation (single-hill) selection for VW resistance in populations derived from 4x × 4x, 4x × 2x, 2x × 4x and 2x × 2x crosses. It was also designed to determine whether family selection would provide more genetic gain than other selection methods, such as individual and within-family selection, in early clonal generations.

To identify VW-resistant clones, several selection criteria, such as maturity, symptom expression, yield and stem colonization, were employed. However, inconsistencies in the relationships among these traits have been reported due to environmental interactions and plant materials (Anderson and Howard 1981, Jansky and Rouse 2000, Frost et al. 2007). In this study, only maturity and symptom expression consistently correlated with each other (Table 1). Although breeders rely on symptom expression for VW diagnosis in the field, results from the present study indicate that symptom expression might be confused with natural senescence, as symptom expression was related to maturity but not stem colonization. Therefore, symptom expression in these populations does not seem to be a good indicator of VW resistance.

The correlation between two tuber generations has been used to estimate early generation selection efficiency in potato breeding (Maris 1988, Bisognin and Douches 2002). Significant correlations between years were observed for yield and symptom expression, but the values were not very high. The correlation coefficient for yield across years was highest in 4x × 2x crosses, supporting previous studies suggesting environmental stability in 4x × 2x families. There was a significant relationship between years for stem colonization in 4x × 2x families. As variation among and within plants occurs for the VW reaction, selecting for low stem colonization in 4x × 2x families may increase the efficiency of selecting for VW resistance in a breeding programme. Low or moderate selection intensity in the single-hill trial may be effective. In addition, it appears that, especially in 4x × 2x families, clones can be selected for yield.

Progeny performance among the four cross types was compared for yield and stem colonization, as yield is an important selection criterion in breeding programmes and stem colonization provides the most accurate disease

Trait	Mean	Single-hill selection			Four-hill selection		
		Individual	Family	Within family	Individual	Family	Within family
RAUDPC	0.4015	−0.133	−0.013	−0.1349	−0.1919	−0.058	−0.1832
Maturity	0.9031	−0.4113	−0.0724	−0.4093	−1.0649	−0.2879	−1.0257
Yield	2.1053	0.2818	0.1321	0.254	0.3033	0.0947	0.2886
LM[1]	1.6207	−0.2181	−0.24	−0.1325	−0.4601	−0.258	−0.3961

Table 5: Expected response to direct selection in single-hill and four-hill trials with a selection intensity = 1.27 (25% of clones)

RAUDPC, relative area under the disease progress curve.
[1]LM: stem colonization mean, log(cfu + 1).

diagnosis. Families from 4x × 4x crosses had the highest yield and stem colonization, while 2x × 4x families had the lowest yield. There are some reports that FDR 2n gamete families are higher yielding than SDR 2n gamete families (Mok and Peloquin 1975, Hutten et al. 1994). The 4x × 2x and 2x × 2x families had high yield and low levels of stem colonization, so these cross types were superior in both years (Table 3). Although clones with some VW resistance were used as parents for each cross type (Bae et al. 2008b), their yield performances were different. Therefore, it is difficult to determine whether differences were due to cross types or parent performances. However, these results suggest that if high-yielding VW-resistant parents are used for 4x × 2x crosses, then a high proportion of superior offspring can be expected.

The single-hill experiment showed higher yield and higher stem colonization than the four-hill experiment. Higher yield in the single-hill families was not expected. However, yield might have been affected by both cultural and environmental differences between the 2 years. Within-row spacing was larger in the single-hill study, reducing competition among clones and potentially contributing to higher yield. In addition, temperatures during the growing season were higher in the second year of the study (four-hill trial). During the four-hill trial, 20 days in July and August were higher than 30°C, but only 2 days were near 30°C in the single-hill trial. This may have reduced yield potential in the four-hill trial.

The single- and four-hill selection strategies provide two different options for early generation in a potato breeding programme. This aspect is distinct from other asexual crop breeding systems, which can make identical trials over a sample of environments, allowing heritability to be measured using intra-class correlation coefficients with continuing years. Therefore, Tai (1974) suggested using the regression type of heritability analysis, estimating in standard units based on the results of analyses of variance for data within a trial and analysis of covariance for data between two trials (single- and four-hill). The other type of analysis uses the variance component, which is measured directly from the results of analysis of variance of the second clonal generation trial. Both results are then compared to determine the genetic advance between selection in the first clonal generation and that in the second clonal generation. These two methods have been used to determine the efficiency of first clonal generation selection for various traits such as maturity, tuber appearance, yield, specific gravity and chip colour (Tai, 1974; Tai and Young 1984). In the current study, the expected response to direct selection in the four-hill experiment had a higher absolute value than that in the single-hill trial. The only exception was for yield using family selection as the phenotypic variation in this group was the lowest. Among the three selection schemes, selection responses were highest for individual selection and lowest for family selection.

Selection progress was observed for disease resistance in the earlier clonal generation, but with lesser gain than direct selection in the second clonal generation. However, considering the cost of operation of a breeding programme, it may still be worthwhile to focus future studies on improving disease expression and measurement in earlier generations. To succeed using early generation selection for disease resistance, distinct disease development in the most susceptible plants is required. Early generation selection for VW resistance is challenging due to ambiguous symptom expression, environmental interactions, and variation in disease levels among and within plants. A simple and accurate screening method for VW resistance would likely improve the effectiveness of early generation selection. For *V. dahliae* a quantitative polymerase chain reaction method has been developed to quantify stem colonization (Atallah et al. 2007). This may provide a good method for identifying resistant clones in segregating populations. In addition, a genetic marker associated with VW resistance allows for the use of marker-assisted selection, which would be expected to improve genetic gain in early generation selection for VW resistance (Bae et al. 2008a). This marker is useful in the populations in which it was tested, and is being used for early generation selection. However, it may not be applicable to all breeding populations. Efforts are underway to generate additional molecular markers for VW resistance.

Based on this study, 4x × 2x and 2x × 2x crosses between parents with high yield and high resistance to VW produce offspring with superior performance and the best opportunities for effective early generation of resistant clones. In the future, it would be interesting to map VW resistance genes in potato to determine whether they are located near the centromere and, therefore, effectively transmitted to offspring via FDR 2n gametes.

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